

CLAIMS

- 1) Live attenuated bacterium of the species *Actinobacillus pleuropneumoniae*, characterised in that said bacterium produces no functional ApxIV toxin.
- 2) Live attenuated bacterium according to claim 1, characterised in that the gene encoding the ApxIV toxin comprises a mutation.
- 3) Live attenuated bacterium according to claim 2, characterised in that said mutation is an insertion and/or deletion.
- 4) Live attenuated bacterium according to claim 3, characterised in that the insertion comprises a heterologous gene.
- 5) Live attenuated bacterium according to claim 4, characterised in that said heterologous gene encodes one or more antigens selected from the group consisting of Porcine Reproductive Respiratory Syndrome (PRRS) virus, Pseudorabies virus, Porcine Influenza virus, Porcine Parvovirus, Transmissible Gastroenteritis virus, rotavirus, *Escherichia coli*, *Erysipelothrix rhusiopathiae*, *Pasteurella multocida*, *Bordetella bronchiseptica*, *Haemophilus parasuis* and *Streptococcus suis*.
- 6) Live attenuated bacterium according to claim 4 or 5, characterised in that said heterologous gene is functionally linked to the promotor region of the *apxIV* gene.
- 7) Nucleotide sequence harbouring the promotor controlling the expression of the *apxIV* gene.
- 8) Nucleotide sequence according to claim 7, characterised in that it comprises the DNA fragment from position 451 to 1132 of SEQ ID NO: 5 or a subfragment thereof still having promotor activity.
- 9) Nucleotide sequence according to claim 7, characterised in that it comprises the DNA fragment from position 617 to 641 of SEQ ID NO: 5.

- 10) Subunit vaccine for the protection of animals against infection with a bacterium of the species *Actinobacillus pleuropneumoniae*, characterised in that said vaccine comprises purified ApxIV toxin and a pharmaceutically acceptable carrier.
- 11) Live attenuated vaccine for the protection of animals against infection with a bacterium of the species *Actinobacillus pleuropneumoniae*, characterised in that said vaccine comprises a live attenuated bacterium according to claims 1-6 and a pharmaceutically acceptable carrier.
- 12) Vaccine according to claim 10 or 11, characterised in that it comprises an adjuvant.
- 13) Vaccine according to claim 10-12, characterised in that the vaccine is in a freeze-dried form.
- 14) Vaccine according to claims 10-13, characterised in that it additionally comprises one or more antigens from pig-pathogenic micro-organisms or viruses.
- 15) Vaccine according to claim 14, characterised in that it additionally comprises one or more antigens selected from the group consisting of Porcine Reproductive Respiratory Syndrome (PRRS) virus, Pseudorabies virus, Porcine Influenza virus, Porcine Parvovirus, Transmissible Gastroenteritis virus, rotavirus, *Escherichia coli*, *Erysipelothrix rhusiopathiae*, *Pasteurella multocida*, *Bordetella bronchiseptica*, *Haemophilus parasuis* and *Streptococcus suis*.
- 16) Method for the protection of a susceptible animal against *Actinobacillus pleuropneumoniae* infection, said method comprising administering a vaccine according to claims 10-15.
- 17) Method for the preparation of a live attenuated bacterium of the species *Actinobacillus pleuropneumoniae* producing no functional ApxIV toxin, characterised in that said method comprises the introduction of a mutation in the gene encoding the apxIV protein.

18) Method according to claim 17, characterised in that said mutation is obtained by introducing a deletion.

19) Method for the preparation of a live attenuated vaccine according to claims 11-15, said method comprising admixing bacteria according to claims 1-6 with a pharmaceutically acceptable carrier.

20) Method for the preparation of a vaccine according to claim 10, said method comprising admixing purified ApxIV toxin with a pharmaceutically acceptable carrier.

21) Diagnostic test for the discrimination between sera from pigs infected with *Actinobacillus pleuropneumoniae* field strains and from pigs vaccinated with a vaccine comprising live attenuated vaccine *Actinobacillus pleuropneumoniae* strains according to claim 1, said test being characterised in that the test comprises purified ApxIV toxin.

19) Diagnostic test for distinguishing *Actinobacillus pleuropneumoniae* infection in pigs from *A. suis* infection in pigs, characterised in that said test comprises purified ApxIV toxin.